# SCIENCE

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## Contents



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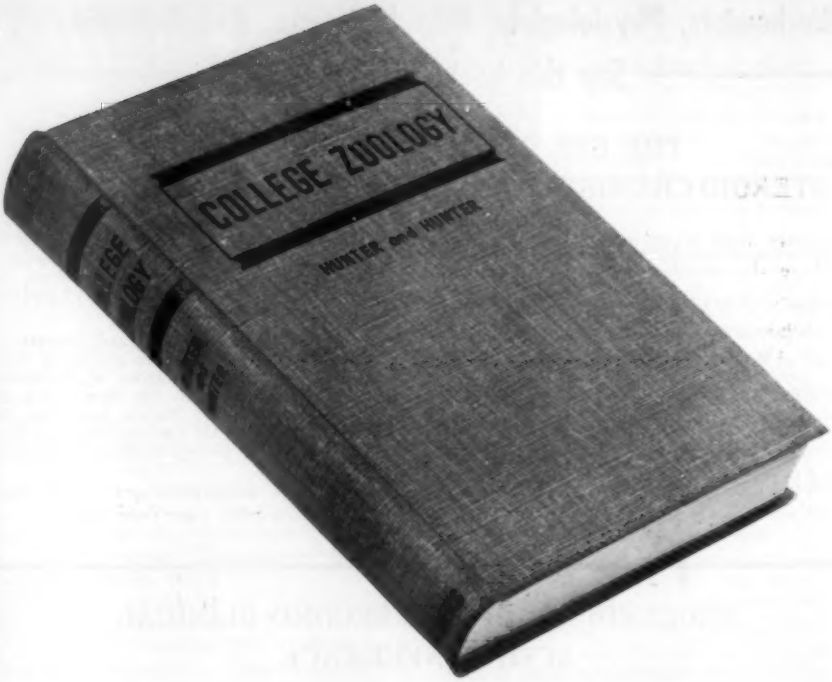


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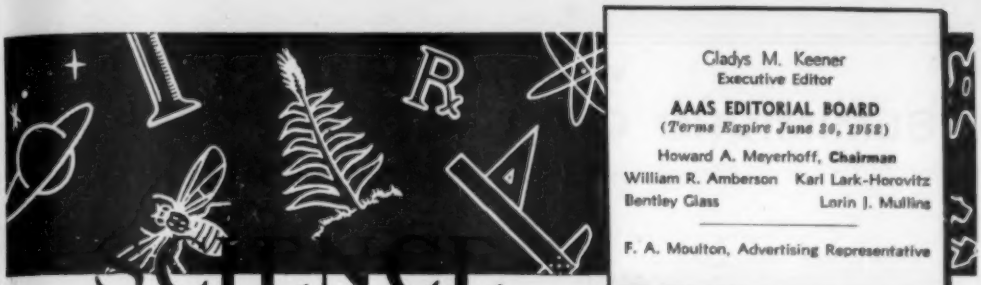




# Quantitative Biology

THE Sixteenth Symposium on Quantitative Biology, sponsored by the Biological Laboratory of the Long Island Biological Association, was held in Cold Spring Harbor June 7–15. Over 300 participants gathered to consider the topic "Genes and Mutations." Many of those attending came from abroad. The following countries were represented: Brazil, Denmark, England, France, India, Italy, Japan, Norway, Scotland, Sweden, Switzerland, Turkey, and Yugoslavia.

Thirty formal papers were presented, and each was followed by an extended and lively discussion. The symposium may be regarded as the most successful of the series of Cold Spring Harbor meetings. The topics, arranged by M. Demerec, director of the Long Island Biological Laboratory, centered around the problems of genetics that have been most intensively studied in recent years. The great changes that have been taking place in the concepts and methods of genetics during the past decade can be well demonstrated by comparing the present symposium with the Cold Spring meeting on the same topic in 1941.

A number of papers were concerned with the modern concept of the gene and its evolution. From different fields, particularly the study of spontaneous mutations, the action of pseudoalleles, and position effects, material has accumulated which is leading toward a revision of the older concept of the gene as a particulate, well-defined unit. Although no agreement exists among the different workers in the field on the extent and kind of revisions necessary, recent developments seem to favor a more flexible and functional description of hereditary units. Among the papers dealing with this aspect of genetics, McClintock's representation of her work on "Chromosome Organization and Genic Expression" aroused particular interest.

One day was devoted to a discussion of cytoplasmic inheritance. The existence of cytoplasmic particles endowed with the ability to reproduce their own kind has been demonstrated for microorganisms, animals, and plants. The interaction of cytoplasmic particles with chromosomal genes in the production of characters was also considered.

In the discussion of induced mutations, the action of chemical mutagens occupied the center of attention. The mutagenic action of ionizing radiations, which had been one of the main topics in the 1941 symposium, was taken up in two papers.

A large part of the symposium was devoted to the genetics of microorganisms. It is becoming increasingly clear that, from a genetic point of view, microorganisms do not behave differently from the higher organisms studied earlier. This was borne out by the demonstration of bacterial chromosomes by DeLamater, by the discussion of the recombination and linkage experiments of Lederberg, and by Witkin's proof that bacterial mutations are nuclear events. For bacterial viruses, mutation and recombination were discussed by Luria and by Hershey. Of especial significance is the discovery of bacterial transforming principles for several characters other than the classical capsular antigens in pneumococci, which was reported by H. Ephrussi-Taylor.

Another topic which led to extended debate was the one-gene–one-enzyme theory of genic action. Horowitz reported an intensive study of temperature-dependent biochemical mutations, which is in good agreement with the theory. Bonner, on the other hand, described experiments demonstrating that many of the biochemical blocks are not absolute, and that the action of one particular enzymatic block may be under the control of several genes. These results seem to require extensive modifications of the original theory.

The symposium ended with a paper by Mirsky on the enzymes found in cell nuclei, and with a discussion by Sonneborn of results obtained by him in *Paramecium* and by Moewus in *Chlamydomonas*, bearing on several aspects of the problems that concerned the participants in the symposium.

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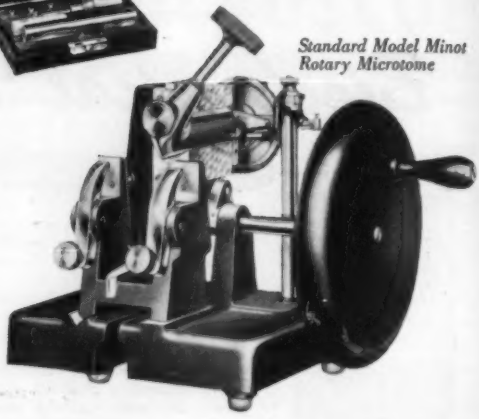

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# A Critical Evaluation of Quantitative Histo- and Cytochemical Microscopic Techniques

David Glick, Arne Engstrom,[1] and Bo G. Malmstrom

*Department of Physiological Chemistry, University of Minnesota, Minneapolis*

THE DEVELOPMENT OF MODERN TECHNIQUES for histo- and cytochemical investigations has placed new tools of wonderful keenness in the hands of medical and biological scientists. The understandable enthusiasm for these fine instruments has, in some instances, been overextended to the point where they have been applied to problems for which they are not fitted, or the results obtained have been interpreted incorrectly through a lack of understanding of their range of reliability. This has happened particularly in the case of microscopic techniques in contrast with the quantitative chemical methods of colorimetry, titrimetry, gasometric analysis, etc., which yield clearly defined histo- and cytochemical data that are much less apt to be misinterpreted. It is our purpose to point out the utility and the limitations of general procedures and approaches that are being increasingly used and misused, in connection with certain of the histo- and cytochemical techniques. These techniques comprise some of the most serviceable and most elegant yet devised, but the keener the tool and the finer the dissection possible, the easier it is to cut one's fingers.

## Errors from Preparation of Material

*Fixation.* Since it is a goal of histo- and cytochemistry to localize and quantitate biologically significant substances and activities, the preparative treatment of the material to be investigated must be considered with respect to its effect on both the localization and quantitation. A very effective way to hold the cellular sample in its original state with regard to these factors is to freeze the material suddenly at a very low temperature immediately upon removal from its source; this can be readily accomplished by immersing the sample in isopentane cooled almost to its freezing temperature (−160° C) by liquid nitrogen. In contrast, the use of fixing solutions cannot as a rule be expected to yield material that has escaped morphological dislocations or concentration changes of chemical constituents. Possibly certain morphological studies might be accurately carried out by subjecting fresh-frozen tissue sections to gaseous fixatives, such as osmic or formaldehyde vapors, while the sections are held at subfreezing temperatures. The use of fixing solutions in studies claiming to reveal true localizations and to yield quantitative data must be considered critically in any specific instance. Although the necessity for fixation in many cases with present techniques is recognized, it would be preferable to avoid fixation whenever possible, and efforts should be directed to improving techniques for observations on living material.

*Dehydration and embedding.* That artifacts and change with respect to cellular topography and chemical composition result from the use of dehydrating liquids on fixed material is probably too well known to merit discussion. The vacuum dehydration of deep-frozen tissue will avoid many of these artifacts and changes.

Unfortunately, the thin-sectioning necessary for the proper observation of morphology requires the use of an embedding medium. The use of any such medium will obviate to some degree the advantages of the freezing-drying preparation, since it involves the introduction of a liquid phase that may cause dislocation and loss of certain constituents. This danger recurs in subsequent removal of the medium from the cut sections with a solvent. The difficulty may be minimal, as in the case of protein structures when paraffin and xylol are employed, or maximal, as in the case of fat structures when these agents are used. The problem could be avoided if a technique were developed that permitted good sectioning, at a suitable thinness, of fresh-frozen tissue, followed by dehydration of the still-frozen sections. Microtoming with a glass knife, which is particularly suited to thin-sectioning (*1*), in a subzero room or in a cryostat cabinet containing dehydration equipment, might accomplish this end, and an investigation of this possibility is now under way.

*Separation of cellular particulates.* The segregation of morphological constituents in cells by centrifugation is being used widely to obtain material for studies relating chemical composition and function to cytological architecture. The separation methods involve the mechanical disruption of cells, followed by differential centrifugations and washings in either aqueous saline or citric acid solutions, or in nonaqueous liquids (e.g., benzene–carbon tetrachloride mixtures). Accordingly, the extraction effect of the medium, as well as its chemical influence on the structural components, must be determined. Control experiments designed to test for such effects should be carried out to determine the reliability of any given procedure. Then, in addition, the possibility of removal of morphologically associated activators and inhibitors of particular chemical reactions during the treatments must also be considered, and appropriate tests, such as recombination of various fractions, should be employed to establish whether these effects are operative.

Of course it is generally true of all the methods of

[1] Rockefeller Foundation fellow.

preparing biological material that problems are posed by liquid treatments and cellular disruptions. Not only are extraction and separation of chemically significant components involved, but also the question of adsorption on interfaces, which either does not occur in the normal living cell but results when the organization of the cell is altered or disrupted by laboratory treatments, or vice versa. For example, does the mast cell granule stain metachromatically with toluidine blue because it contains heparin intrinsically, or because the substance is adsorbed on the granule surface from the cytoplasm as a result of the preparative treatment? Furthermore, does the fact that most of the heparin is present in the final supernatant after larger particles and microsomes are spun out (*2*) result from extraction of the heparin from particulate matter during the laboratory treatment, or because the compound is dispersed in the interparticulate phase *in vivo*? It is thus imperative to make interpretations sufficiently liberal to encompass all possibilities until more definite information is at hand.

It was pointed out by Potter (*3*) that a test can be made for contamination of separated particulates by other cellular material. Use is made of the principle that different particulates can be characterized by their content of specific substances or activities—e.g., desoxypentose nucleic acid for nuclei. Thus, the presence of desoxypentose nucleic acid in mitochondria would be taken to indicate contamination by nuclear or whole-cell material. In setting up biochemical criteria by which a particulate is to be characterized, it would contribute to reliability to establish by a method other than one involving centrifugal separations from homogenates that the biochemical properties in question are confined to the given particulate. This has been done in the case of desoxypentose nucleic acid in nuclei, since evidence for this localization has come not only from centrifugal separations but also from Feulgen studies and ultraviolet absorption observations. The possibility remains that chemical components other than the one or more tested may contaminate the particulate by some means, such as selective adsorption during the cellular homogenization, so that a morphologically pure fraction may still be biochemically impure with respect to the *in vivo* composition.

## Errors in Chemical Staining Techniques

The use of staining reactions in histo- and cytochemistry contributes to the greatest volume of published work in the entire field. Both anatomists and pathologists in particular have been quick to appreciate the manifold advantages of endowing the ultimate biological structures with the significance of their chemical constitution and function. It might be of interest to set up some warning posts along the diverse paths that cross this area.

*Specificity.* The chemical specificity of any staining reaction must be clearly defined for it to be of service rather than disservice. That certain chemical groups are responsible for certain staining reactions is quite often beyond controversy. The difficulty arises when different chemical groups react with the same reagent —e.g., as in silver stains—or when the same reactive group occurs in different compounds—e.g., aldehyde visualized by Schiff's reagent. In such cases additional evidence must be presented to establish the claim that a stain has resulted from the occurrence of a particular compound or class of compounds. When this evidence is insufficient, no end of discussion is apt to develop concerning the relative merits of one possibility or another. It would be better to reserve judgment in these cases until positive conclusions can be justified. Some examples of the difficulties which develop when the chemical specificity of stains is open to variable interpretations may be found in a recent paper by Gomori (*4*).

*Stoichiometry.* It is obvious that the quantitation of a cellular substance *in situ* by a staining reaction requires that the reaction be a stoichiometrical one. The following consideration is given to the chances of achieving this end.

A staining reaction can be expressed by the following equation:

$$D + S \rightleftharpoons DS,$$

where $D$ is the dye, $S$ its substrate, and $DS$ the stained substrate. It will be assumed that the stain is specific so that there is only one substrate for $D$. Applying the law of mass action one has:

$$\frac{(DS)}{(D)(S)} = K.$$

If $K$ is very large the reaction can be written as follows:

$$D + S \rightarrow DS;$$

i.e., it is irreversible. In this case all of $S$ will react with $D$ to give $DS$, as long as $D$ is present in excess. The excess $D$ can be removed by washing without dissociating $DS$. Thus, the amount of $DS$, which is the quantity that is determined photometrically, will be directly proportional to the original amount of $S$. This would be the ideal case for a quantitative reaction.

If $K$ is not very large, the following relation holds:

$$(DS) = K(D)(S).$$

It should be pointed out that this does not mean that $(DS)$ is directly proportional to the initial concentration of $D$ and the amount of $S$ present in the section, since $(D)$ and $(S)$ are equilibrium, not initial, concentrations. The discussion will be simplified if it is assumed that $(D)$, initial, is very great, so that the reaction with $S$ will not appreciably decrease it—i.e., $(D) \simeq (D)_i$. We then have:

$$(DS) = K(D)_i(S) = K'(S).$$

Since $(S) = (S)_i - (DS)$, it follows:

$$(DS) = K'[(S)_i - (DS)], \text{ or}$$

$$(DS) = \frac{K'}{1 + K'}(S)_i.$$

This equation shows that, under the conditions assumed in the derivation, $(DS)$ is directly proportional

to $(S)_4$. It would then seem that a reversible reaction would be also satisfactory from a quantitative standpoint, if only the dye were present in a large excess. There is, however, one serious complication: the excess dye must be removed by washing, and even under standard conditions it will be difficult to attain a final $(DS)$ that reflects the initial amount of $S$.

Although a strongly irreversible reaction would be ideal, a reversible reaction would be satisfactory if the dye were present in large excess, and if $K$ were still relatively large. Other limiting factors, such as adsorption, diffusion, and imperfect accessibility of reagents to particular sites because of structural barriers, also contribute to the impossibility of satisfying the conditions for quantitation of a reversible reaction *in situ*. These factors can operate even in the case of an ideal irreversible reaction, and the aberrations arising from them are, for the most part, indeterminate. Hence, the chances for reliable quantitation by staining reactions are rigidly limited, although not necessarily eliminated, even when the reaction meets the requirements of specificity, and stoichiometry is obtained in the test tube.

Many of these difficulties are likewise encountered in purely morphological studies, in which the requirements for reliability are less rigorous. In this connection Gomori (*5*) and Novikoff (*6*) have discussed some of the errors involved in the special case of enzyme-staining.

*Chemical composition.* When a chemically complex substance, such as a protein, is to be determined by the measurement of one of its constituents, such as tyrosine, it is assumed that the constituent represents a constant fraction of the complex molecule. It cannot be assumed, however, that the composition of the complex substance in one cell or body fluid is identical with that to be found in other anatomical compartments in the same organism. Thus, the proportion of tyrosine in a serum protein cannot be taken to be the same as that in the protein of a liver cell nucleus unless experimental proof is supplied. In the case of tyrosine, its content in serum globulin has been reported as 6.2 per cent, in serum albumin 4.8 per cent, and in liver nucleoprotein 3.6 per cent (*7*). Of course all the tyrosine need not be in protein, and this too should be taken into account.

Another variable to be considered with respect to chemical composition is the effect of the degree of polymerization of certain dyes when bound to certain substances (*8–10*). This process will change the absorption spectrum of the dye, the magnitude of the change depending on the degree of polymerization. The latter will depend on the relative amount of the dye with respect to the amount and chemical nature of the substrate. The first factor will result in a wavelength shift that will vary with the substrate concentration, and thus large deviations from Beer's law will result. A metachromatic reaction would, therefore, not be suitable for photometric analysis in the usual manner—i.e., absorption measurements at a fixed wavelength. It is possible that some quantitative information might be obtained by measuring the degree of metachromasia—i.e., the magnitude of the shift of the absorption maximum, since this depends on the degree of polymerization, which in turn bears some relation to the substrate concentration.

## Errors in Microspectrography

The physical errors occurring in the measurement of any kind of electromagnetic radiation used for analytical purposes can be grouped into two main classes: errors in the instruments and measuring technique used, and errors due to certain properties of the objects to be analyzed. When electromagnetic radiation is used for histo- and cytochemical analysis, the two groups of errors are considerably more difficult to master than in the case of absorption measurements on a liquid in a spectrophotometer. The theory and design of proper instrumentation for quantitative cytochemical analysis have been described for ultraviolet and visible light by Caspersson (*11, 12*) and Thorell (*13*), and for x-rays by Engström (*14*). A general treatment of optical methods used in biology appears in the *Transactions of the Faraday Society* (Sept. meeting [1950]). In the present survey only physical errors inherent in the biological object will be discussed.

*Validity of the Lambert-Beer law.* In biological systems such as parts of cells or tissues, there are areas having relatively high absorptions of radiation, which may be either natural or induced by staining. The first question that arises when radiation is passed through a sample for analytical purposes is whether the Lambert-Beer law holds. In other words, is the extinction of the radiation proportional to the product of concentration and thickness of the absorber? For many substances the Lambert-Beer law can be tested in macromodel systems. The unique conditions existing in thin slices of tissue having a high absorption make such macromodel tests less applicable. The test for the validity of the Lambert law can, for present purposes, be performed by measuring the absorptions by sections of different thicknesses of the *same homogeneous* tissue or cell.

The effect of the orientation of the molecules on the absorption has been brought up as a cause of deviation from the Lambert-Beer law, especially when the amount of nucleic acid is determined at the absorption maximum of 260 mμ (*15*). The reason for the deviation is that light incident to the absorber will be absorbed differently, depending upon the planes of polarization in relation to the orientation of the molecules. It has recently been shown by Ruch (*16*) and Ruch and Thorell (*17*) that the dichroism of fibers prepared from a pure solution of desoxyribonucleic acid is very high; extinction values of over 3 have been recorded. If the nucleic acids were oriented to that degree in tissues, large deviations from the Lambert-Beer law would result when nonpolarized radiation is used for the absorption measurements. However, in structures where a strong orientation might be expected, such as in giant chromosomes from *Chiro-*

*nomus* or *Drosophila* and in muscle fibers, the actual ultraviolet dichroism observed is low, and the error is negligible when nonpolarized light is used for the absorption measurements.

*Relative homogeneity of absorbing material.* Other, greater errors occur in the absorption measurements. One of the most intriguing properties of the cell, or of biological tissue in general, is the inhomogeneous distribution of absorbing substance. When parallel x-rays are used for the estimation of cellular components, the inhomogeneities along the beam are superimposed, and thus only the inhomogeneities in the plane perpendicular to the beam have to be considered. When a microscopical system is used for micromeasurements of absorption, the situation is much more complicated because of the biconical shape of the beam. The actual depth of focus in a high-power optical system is of the order of magnitude of about 0.5 μ. Thus, inhomogeneities along the axis of the beam must be considered also, if histological sections of the usual thicknesses are used.

A simple estimate of the effect of the inhomogeneous distribution of an absorbing substance in one plane perpendicular to the beam gives the following result: The area under analysis is assumed to be unity, and the intensity of the incident radiation ($I_o$) is uniform over this area. If there is a homogeneous distribution of absorbing material of concentration $c$ and thickness $b$, the intensity of the transmitted light ($I$) can be calculated from the Lambert-Beer law:

$$I = I_o e^{-kcb}, \text{ where } k \text{ is a constant.}$$

If, instead of being uniformly distributed, the absorbing material is concentrated in a fraction, $\frac{1}{q}$, of the total area, there will be an area $(1-\frac{1}{q})$ that is nonabsorbing. The following expression then gives the intensity of the transmitted light:

$$I_q = I_o(1-\frac{1}{q}+\frac{1}{q}e^{-kcqb}).$$

In practice the concentration of absorbing material is assumed to be proportional to the optical density. The magnitude of the error due to nonhomogeneity is therefore apparent if the ratio of the optical densities in the two cases ($D$ and $D_q$) is calculated:

$$\frac{D}{D_q} = -\frac{0.434\,kcb}{\log\,(1-\frac{1}{q}+\frac{1}{q}e^{-kcqb})}$$

Fig. 1 gives the value of this ratio as a function of $q$ for a number of different values of $kcb$. The ordinate tells how many times too small the concentration calculated from the absorption measurement is, compared to the true concentration. It is obvious that for any type of accurate absorption measurement the area under analysis must be quite homogeneous. One way to get greater accuracy is to carry out the measurements at a very high resolution and integrate them over the area being analyzed. In the determination of the amount of absorbing substance in a nucleus, for example, this procedure of arriving at a relatively correct value is exceedingly time-consuming. If a whole nucleus is measured, the errors depending on the inhomogeneous distribution become greater. In fact, all measurements of biological objects that are more or less inhomogeneous have a greater or smaller error that can be roughly estimated. The important point is that the measurements contain an inherent error that must be taken into account when interpreting the results.

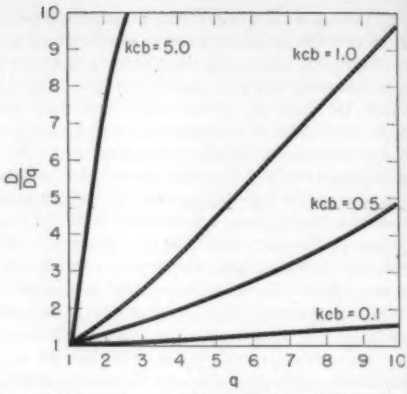


FIG. 1. Ratio of the optical density in a homogeneous optical field ($D$) to that in an inhomogeneous field ($D_q$) as a function of the degree of inhomogeneity, in terms of the number of times ($q$) reduction in the area containing the same amount of the radiation absorbing material.

*Range of extinction coefficients.* In some papers dealing with absorption measurements of biological material, extinctions as high as 2 (i.e., 1 per cent transmission) have been reported. If the error in estimating the transmission is assumed to be constant, the error in concentration will vary depending on the exponential absorption of the light, provided the Lambert-Beer law holds.

The following formula has been derived by Ringbom (*18*):

$$\frac{\frac{100\ dc}{c}}{dI} = -\frac{43.4}{\rho I}.$$

This expression gives directly the relative analytical error caused by an absolute photometric error of 1 per cent, when $I_o$ is set at a transmission of 100 on the galvanometer scale. The magnitude of this error as a function of the transmission is given in Fig. 2. The curve in Fig. 2 is obtained on the assumption that $dI$ is 1 per cent over the entire transmission range. Since the value of $dI$ may vary with the instrument used, and even with the same instrument if a switch is included to change the range of sensitivity, as in the Beckman spectrophotometer, the curve in Fig. 2 must be interpreted accordingly. For example, if $dI$ at a given transmission value is 0.5 per cent, the error would be half that indicated in Fig. 2. The best results in absorption measurements will be obtained in the range of 20–60 per cent transmission, which corresponds to an extinction range of 0.7–0.2.

A discussion of photometric error has appeared in recent papers by Cole (*19*) and Robinson (*20*), and a subsequent note by Cole and Robinson (*21*) indicates areas of agreement and a correction of some overgeneralization in the paper of Cole (*19*).

*Nonspecific losses of radiation.* A factor that complicates many absorption measurements is the scattering of radiation. In the x-ray region the scattering is only a fraction of a per cent of the absorption and therefore completely negligible. In the visible and ultraviolet region, however, the increased scattering can be a highly complicating factor when quantitative measurements of biological material are desired. There are very great difficulties in estimating the radiation scattering in parts of cells, etc. (*22*). Attempts at applying Rayleigh corrections in the ultraviolet region, based on measurements at longer wavelengths, cannot be very successful, since the magnitude of the exponent of the wavelength cannot be predicted. It is also difficult to estimate the proportions of the inherently reflected and scattered radiation.

*Thickness of radiation absorbing structure.*[2] Finally, another complicating factor should be mentioned. Depending on the different methods used to prepare samples for microspectrography, the thickness of the absorbing layer may vary from point to point in the sample. In comparing amounts of substances in different parts of cells the thickness must be known. At present there is no easy method for a very exact determination of the thickness of ordinary histological sections. The usual technique of focusing with a microscope on the upper and lower surfaces may not be reliable, since the movement of the tube is not a linear function of the fine screw motion in many microscopes. For a high-power optical system, NA 1.30 and a total magnification of 1,000, the depth of focus is about 0.5 μ (*24*). In estimating the thickness of a section, the maximal error in this case would then be 1.0 μ. The error can be diminished by performing a great number of measurements and treating the results statistically.

Interference methods have also been proposed for measuring the thickness of microtome sections. For that purpose flat surfaces are required, and these are found only in the tissue-free areas of embedded sections. The thickness of the section at a tissue-free area, however, may not be the same as that at the point where the structure to be investigated is found. For structures lying near the edge of the section and adjacent to the tissue-free edge of the embedding medium, the method may be applicable. Furthermore, absorption measurements of x-rays, α-rays, or β-rays may be used in a tissue-free area of the section for the determination of thickness, but these methods will have the same limitations as the interferometric procedure.

Shadow-casting at oblique angles can be used for the determination of the thickness of a specimen close to the edge of a section, but not for structures near the center. For isolated cells or structures, as in blood smears and squash preparations of chromosomes, shadow-casting can be utilized for the determination of thickness. However, the necessity of performing the shadow-casting in high vacuum excludes the use of the method for living cells.

Thickness measurements may be avoided altogether if the results of absorption measurements are referred to dry weight per unit area, as determined by x-ray absorption.

This discussion has dealt only with physical errors in microspectrography, but one important source of error of a chemical nature should be pointed out, namely, the error resulting from the effect of certain radiations on cellular compounds to be analyzed (*25*, *26*). For example, ultraviolet absorption by solutions of pentosenucleic acid decreases with time because of the breakdown of the molecules by radiation. In any given case it would be necessary to establish that the time of exposure to the radiation during measurement was such as to confine the error to an allowable known range.

There is an attitude prevalent in some circles that, although spectrophotometric measurements on stained tissue admittedly cannot give data regarding absolute quantities of chemical constituents in given cases, they still may offer reliable "semiquantitative" or relative values. This would be acceptable if the order of magnitude of the error were established by objective means and proved to fall within justifiable limits.

From the foregoing it is apparent that uncertainties exist in both the attempts at localization and quantitation of chemical constituents in cells and tissues. Errors arise from the artificial conditions imposed by fixation, dehydration, and embedding and staining procedures, as well as from those attending the techniques used for the separation of cellular

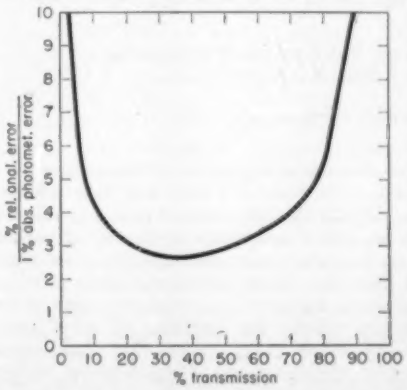


FIG. 2. Relative analytical error for an absolute photometric error of 1 per cent as a function of the percentage of transmission.

[2] After this manuscript was submitted for publication a note by Glimstedt and Hakansson (*23*) appeared on the mechanical measurement of thickness in histological sections.

particulates, but within limits these errors can be minimized. The questions of specificity, stoichiometry, and variable chemical composition as applied to staining reactions must also be considered with respect to the errors of quantitation *in situ* that may result from these factors.

With reference to errors inherent in microspectrography as practiced in histo- and cytochemistry, the validity of the Lambert-Beer law does not appear to be threatened by the degree of orientation of the molecules in most biological materials. However, the relative inhomogeneity in the distribution of absorbing substances introduces an error that can be significant. Reliable measurements are possible only in a well-defined extinction range, and nonspecific losses of radiation in some cases can be serious in the visible and ultraviolet region. The determination of the thickness of a cellular sample for analytical purposes likewise presents difficulties, and the chemical effects of radiation used for absorption measurements cannot be ignored.

From these considerations, it appears unwise to accept, too literally, the localizations of chemical substances in finer cellular structures as indicated by staining reactions. Furthermore, little reliability can be expected from attempts at quantitation *in situ* by means of microspectrography of stained structures in much biological material. Reliable localizations of chemical substances can be obtained in unstained material by microspectrography in the ultraviolet and x-ray regions. However, the errors of inhomogeneous distribution of the radiation-absorbing substance, nonspecific radiation losses, and chemical effects can gravely interfere with quantitative work in the ultraviolet. X-ray absorption, as it has been employed, is affected less by these factors, and its use can yield reliable quantitative data, as well as true morphological patterns.

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# *Technical Papers*

## Oxygenation of Blood by Isolated Lung


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Recent progress in intracardiac surgery has necessitated the diversion of blood from that side of the heart, usually the left, involved in the operative field. This has been accomplished by the use of mechanical pumps (*1*), which have short-circuited blood from the area. However, future advances in major intracardiac operative procedures—i.e., repair of septal defects—may well involve the diversion of blood from all chambers of the heart (except that to the coronary vessels). Although most of the technical difficulties governing the artificial propulsion of blood have been overcome, that of insuring adequate oxygenation persists.

Previously proposed methods of oxygenating blood *in vitro* in an extracorporeal blood circuit involving either mechanical mixing of blood with oxygen (*2*) or intravenous introduction of chemical oxidants (*3*) have proved unsatisfactory.

Although older isolated heart-lung preparations as a rule operated far below physiological efficiency, pulmonary oxygenation was well maintained. It occurred to us, therefore, that an isolated donor lung, functionally attached to a mechanical propulsive unit, would successfully oxygenate venous blood pumped through it without the disadvantage of the foaming or hemolysis of red blood cells associated with the use of artificial oxygenators.

To verify this point, the heart and lungs of a cat were removed following death by exsanguination. No attempt was made to maintain sterility of the operative field. As previously described by one of us (*4*), the pulmonary artery and veins of one side were


[1] The authors wish to express their appreciation to A. S. Buchberg for his technical aid.

cannulated and the opposite pulmonary vessels tied off. A tracheal cannula was inserted and attached to a source of oxygen under intermittent positive pressure. The animal's blood was placed in a reservoir kept under slight negative pressure by the action of a rotary pump (*5*), and circulated through the isolated lung, which was inflated 12 times/min by positive pressure. No effort was made to keep the lung at body temperature. High oxygen content of the blood was promptly achieved and maintained for periods of 60–90 min.

To test further the ability of the removed lung to oxygenate blood, the circulating system was stopped and the blood replaced by intensely cyanotic blood from another animal. The pump was then started again, and blood samples withdrawn from the pulmonary veins at 5- to 10-sec intervals. These were analyzed for oxygen content by the Scholander syringe method. The results of two experiments are graphically depicted in Fig. 1.

From the results shown, it is evident that the blood was completely oxygenated during a single passage through the isolated lung. In the case of the second procedure illustrated, the reservoir was kept at an abnormally high negative pressure, resulting in gross pulmonary edema sufficient to produce considerable frothy secretion in the tracheal cannula. Despite this limitation, oxygenation, although less complete than usual, was rapidly achieved and maintained.

The results indicate that the isolated lung, up to 90 min after removal from the body, and not subjected to any special care or treatment, is capable of oxygenating blood rapidly flowing through it. Certain limitations remain as yet undetermined. These include the longest interval following removal during which function is retained, the maximal blood flow permitting efficient oxygenation, and the maximal duration of function under mechanical propulsion. Whether the isolated lung releases any noxious substances that may injure an intact animal is as yet unestablished.

Further application of this form of oxygenation to experimental, and presumably clinical, surgery involves several hemodynamic considerations. It is questionable whether a single propulsive mechanism will maintain normal pressures and flow through the greater circulation and allow sufficient residual pressure to maintain adequate flow in the pulmonary bed, especially since aeration is accomplished by positive rather than negative pressure. Since a second pump may be necessary, analogous to the two sides of the heart, the problem of maintaining equal outputs of the two pumps will arise.

These factors are of future concern. Of interest is the fact that a relatively simple use of a natural oxygenator—an isolated lung—may make possible the diversion of blood from the heart. For the physiologist, it may permit more efficient perfusion of isolated organs for the investigation of their functions.

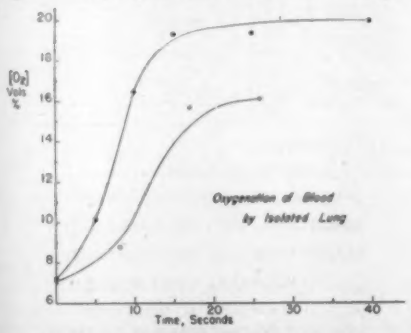


FIG. 1. Rapid oxygenation of deeply cyanotic blood circulated through an isolated lung receiving oxygen under intermittent positive pressure. ●—Expt. 1; ○—Expt. 2.

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## Duration of Action of Residual DDT Deposits on Adobe Surfaces


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Observations made in different countries on the effectiveness of DDT residual deposits in anopheline control reveal that far from uniform results are obtained by different workers. Causes for this lack of uniformity may rest in several factors. The mosquito species being studied is undoubtedly an important factor, or at least a confusing one, since species differ markedly in habits, including house-resting habits, and also possibly may differ in response to minimal exposures to DDT. Observations of Muirhead-Thomson (*1, 2*) with *Anopheles gambiae*, for example, do not parallel observations of Wharton and Reid with *A. maculatus* (*3*), Swellengrebel and Lodens with *A. aconitus* (*4*), Bertram with *A. minimus* (*5*), or Downs and Bordas with *A. pseudopunctipennis* (*6*). Another factor of undoubted importance is the surface on which the DDT is being sprayed. Clapp *et al.* (*7*), Sundararaman and Peffly (*8*), and Maier *et al.* (*9*) have shown that surfaces of different construction materials commonly used in the tropics will retain DDT activity for varying periods of time.

Soil, as sun-baked adobe bricks, or as a plastering mixture of wet soil alone, or wet soil mixed with substances such as straw or manure, and applied over a wall of woven reeds or branches (*bajareque*) is a very

[1] The studies and observations on which this paper is based were conducted under the auspices of the International Health Division of the Rockefeller Foundation with the cooperation of the Ministry of Health of Mexico.


[2] Grateful acknowledgment is made of the help and advice given by J. Pitner and other personnel of the Soils Laboratory of the Rockefeller Foundation Agricultural Program in Mexico in the chemical analyses of the soil samples, and to R. Bradfield for suggesting the technique for determination of Fe in the form of free oxides.

common construction material in the tropics. Clapp *et al.* (*7*), using panels made from an alluvial clay, and Sundararaman and Peffly (*8*), using panels made from clay-cowdung mixtures, noted that the DDT rapidly lost its effectiveness on such a surface, and observations from many regions of the world, including those of Muirhead-Thomson in West Africa (*2*), Maier *et al.* in Venezuela (*9*), and Gaud *et al.* in Morocco (*10*), indicate that they observed a loss of DDT activity in a period of a few weeks or months. Puri in India (*11*) noted that the loss of activity may vary considerably in different regions of the same country.

Earlier observations reported from Mexico by Downs *et al.* (*12*) showed that with some adobes there was evidence of persistence of DDT activity for a period of years, and results of investigations of this problem are here reported.

In our experiments, adobe bricks from different regions of Mexico were obtained. These bricks contained added straw or sand reinforcement as used in the region. Four soils were selected, one from Mixquic, Distrito Federal, derived from lake-bottom loamy soil with high organic matter content; one from Acatlipa, Morelos, of sandy clay; one from the delta of the Rio Coyuca, Guerrero, of deltaic deposit, and one from Tuxpan, Michoacan, of a red, clayey soil. All these regions are malarious. DDT water-wettable powder (Santobane #50 W, Monsanto Chemical Company) was sprayed on the bricks at a rate of about 200 mg DDT/sq ft. The approximate amount sprayed on each brick was determined by quantitative chemical analysis (using an alkali dehydrochlorination method) of deposits on filter papers placed at each side of the brick. For the biological tests colony-reared *A. aztecus* and *A. albimanus* were employed. Some 15 mosquitoes, anesthetized with $CO_2$, were placed on the surface to be tested, under a Petri dish containing a wad moistened with sugar solution, and left for exposure periods of 15 min and 1 hr. After exposure the mosquitos were again anesthetized and transferred to a clean sheet of paper under a Petri dish with a wad of sugar solution, and were held for 23 hr. Mortality was recorded at 15 and 30 min and at 1, 2, and 24 hr after exposure. All tests were rigidly controlled, both with parallel runs on unsprayed bricks and with runs on cloth panels treated with accurately determined deposits (10, 25, 50, and 100 mg/sq ft) of 75% *para-para'* isomer of DDT, deposited on the cloth panels from an acetone solution. Duplicate runs were made at each testing period.

Soil analyses were made as follows: pH determined with Beckman potentiometer on a fresh 50% water suspension of soil; soluble chlorides by washing a 10-g sample of soil with hot water several times in a Buchner funnel and determining the chlorides by the Volhard method; base exchange capacity using the technique of the Association of Official Agricultural Chemists (*13*). Total calcium and total aluminum were determined after alkaline fusion. Fe was determined by three different procedures: first, after alkaline fusion, to determine total Fe; second, after hot concentrated HCl extraction, to determine total extractable Fe present in the form of oxides and hydrated silicates; and, third, a method using nascent hydrogen reduction in a concentrated oxalate medium, as described by Jeffries (*14*), to determine Fe present in the form of oxides only. Soils extracted by the latter technique were tested for catalytic dehydrochlorination of DDT and were found to have lost about 90% of their catalytic activity.

The technique for determining the breakdown of DDT by different soils is a modification of the technique developed for determining catalytic dehydrochlorination of DDT by Fleck and Haller (*15*). Measured amounts of DDT and soil are placed in a U-tube, immersed in an oil bath at 130° C, and clean, dry air blown across and collected in a beaker of water, agitated by a mechanical stirrer, and with phenolphthalein added as indicator. Decinormal solution of KOH is added from a burette, and the evolution of HCl plotted against time. A detailed report of this technique will be published at a later date.

Results of biological tests are summarized in Fig. 1. It is evident that there is a very marked difference in duration of effectiveness of the DDT residual deposit. Soil from the Distrito Federal has allowed the DDT to retain a high degree of activity for nearly 3 full years, and is still active at time of this writing. Soil from Morelos held activity for a year and more, whereas the soils from Guerrero and Michoacan inactivated the DDT in 3–6 months.

When these soils were tested to determine their activity in catalyzing the breakdown of DDT (75% *para-para'* isomer) at 130° C, the same relationships were observed (Fig. 2). The soil from the Distrito Federal does not initiate a reaction at all in 3 hr; the

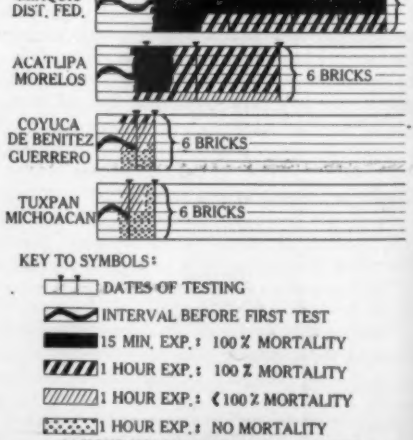


FIG. 1. Duration of action of residual DDT on adobe surfaces.

soil from Morelos initiates a reaction which decomposes about 10% of the DDT; the soil of Guerrero, a reaction which decomposes about 20% of the DDT; and the soil from Michoacan, a reaction which decomposes about 90% of the DDT. All evidence to date shows that the undecomposed residue of DDT remains active, thus indicating that a soil can be saturated, and after this saturation point is reached, undecomposed DDT remains and may accumulate with repeated sprayings. Experiments using varying proportions of DDT and soil will be reported in a later publication.

Chemical analyses of the soils (Table 1) reveal that those soils which catalyze the decomposition of DDT most effectively are the soils highest in Fe and Al. Alkalinity of the soils would not appear, from these results, to be a significant factor in catalyzing the DDT breakdown, nor does it appear to be related to adsorptive properties of the soils, as measured by the base exchange capacity.

Fleck and Haller (*15*) showed earlier that Fe and Al catalyze the decomposition of DDT. It is reasonable to assume from the above data that these substances, in complex and active form in the soil, are responsible for the phenomenon here reported, although the action of other, undetermined substances is not necessarily excluded.

Tests run in our laboratory with $FeCl_3$ and $AlCl_3$ added to the DDT in the reaction tube indicate that $FeCl_3$ catalyzes the decomposition of DDT at a very much faster rate than $AlCl_3$, and that for a given concentration of catalyst, $FeCl_3$ will decompose much more DDT than will $AlCl_3$. Apparently, therefore, Fe in the soil plays a much more important role than Al in catalyzing the decomposition of DDT. Tests using $FeCl_3$ have shown that very small amounts of Fe are necessary for the reaction (1 molecule of Fe will catalyze the decomposition of approximately 160 molecules of DDT, the reaction finally stopping, apparently as a result of accumulation of DDT decomposition products). In the case of soils, we have found that 50 mg of soil (Guerrero #1) will decompose only 1.0 mM of DDT, whereas the total amount of Fe present in the same soil should account for approximately 30 times this amount. It is therefore our deduction that only a small fraction of the Fe present in the soil is available in active form to catalyze this reaction. The figures for Fe in the form of oxides more closely parallel the data on catalytic activity of soils than do the figures for total Fe and for hot HCl extractable Fe. This, plus the observation that soils extracted by the method of Jeffries have lost most of their catalytic activity, leads to the conclusion that it is the readily available iron oxide fraction of the soil that is responsible for the catalytic activity.

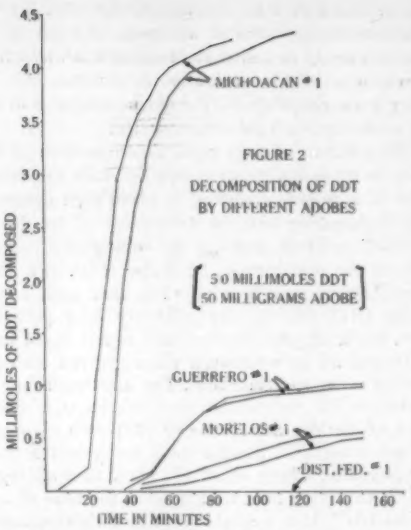


FIGURE 2

DECOMPOSITION OF DDT BY DIFFERENT ADOBES

FIG. 2. Decomposition of DDT by different adobes.

Demonstration of the marked variability in the activity of different soils in catalyzing the decomposition of DDT may well help to explain some of the divergent reports on DDT action appearing in the literature.

The method for determining the reactivity of different soils is of potential importance in the rational planning of malaria control campaigns. A given soil

TABLE 1

CHEMICAL ANALYSES OF SOILS FROM DIFFERENT REGIONS OF MEXICO*

| Soil | Humidity (per cent) | Loss on calcination (per cent) | pH | Base exchange capacity (mEq/100 g) | Soluble chlorides (per cent Cl−) | Silica (per cent $SiO_2$) | Total Ca (per cent) | Total Al (per cent) | Fe | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Total (per cent) | Hot HCl extract (per cent Fe) | Extractable oxides (per cent Fe) |
| Distrito Federal #1 | 9.67 | 17.2 | 8.33 | 48.0 | 0.06 | 42.9 | 3.67 | 9.9 | 7.13 | 1.8 | 0.74 |
| Morelos #1 | 4.66 | 4.5 | 7.45 | 16.4 | 0 | 54.6 | 1.75 | 6.67 | 17.0 | 2.54 | 1.29 |
| Guerrero #1 | 1.11 | 5.02 | 8.30 | 16.7 | 0 | 39.2 | 0.71 | 12.7 | 19.2 | 5.7 | 1.65 |
| Michoacan #1 | 6.21 | 11.3 | 6.75 | 17.2 | 0.004 | 41.5 | 2.57 | 7.2 | 25.7 | 11.5 | 9.29 |

* All percentages are referred to dry weight of soil.

can be tested in 3 hr, eliminating the need for more time-consuming chemical analyses, and up to four soils can easily be tested at the same time, by suitable arrangement of the apparatus. At present, soils from every town receiving DDT residual spraying in Mexico are being analyzed and classified.

The problem of very rapid decomposition of DDT when in contact with some soils demands the development of a practical method to avoid such decomposition. Laboratory tests we have made of the duration of DDT residual deposits on whitewashed surfaces confirm the observations of Maier *et al.* (*9*) and of Hadjinikolau and Busvine (*16*) that such surfaces retain DDT activity for relatively long periods of time. Hadaway and Barlow (*17*) report rapid loss of DDT activity on whitewash when sprayed with kerosene solutions and emulsions. The whitewash they used contained Fe (calculated as 2.6% $Fe_2O_3$). Further work of Barlow and Hadaway (*18*) with suspensions of water-wettable powder gave much better results. Furthermore, Clapp *et al.* (*7*) show that adding salt to the whitewash mixture lengthens the time of action of the DDT. It is probable that Maier's recommendation that walls be whitewashed before being sprayed with DDT will provide a solution to the problem presented, provided that whitewashes with low Fe content are used. A field trial of this hypothesis is now under way at Tuxpan, Guerrero. A search is also being made for substances which, when mixed directly with the DDT suspensions in the spray tanks, may inhibit the rapid decomposition of the DDT through inactivation or blocking of the catalyst, and still be cheap enough to be used in control campaigns.

Here it may also be remarked that DDT-kerosene solutions applied to adobe not only will carry the DDT in solution deeper into the adobe, out of effective range as a contact insecticide, but will also place the DDT in much closer contact with the Fe in the adobe than when DDT suspensions are used. This may help in clarifying further the relative inefficiency of such DDT-kerosene solutions, reported earlier by Barlow and Hadaway (*18*).

The reported observations may also have a bearing on problems presented by the use of DDT in agriculture. Detoxification of DDT-poisoned soils by dilute $FeCl_3$ solutions is a possibility.

*Addendum*: Since this manuscript was submitted for publication a paper describing the change in physical status of DDT applied to mud surfaces has appeared (*19*). We have also observed this phenomenon, including the loss of DDT activity biologically long before it disappears chemically. We feel that sorption is the first step in the process of catalytic decomposition. Sorption, in soils high in iron, takes place very rapidly (in Michoacan #1, 7 days for a 250 mg/sq ft deposit of DDT), and the reaction of catalytic decomposition at normal environmental temperatures delays for weeks or months. The reaction of catalytic decomposition at 130° C affords a rapid method for foretelling the capacity of a soil for inactivating DDT.


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## A Simple Humidifying System for a Small High-Humidity Room[1]


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*The George Washington Carver Foundation* and *the School of Mechanical Industries, Tuskegee Institute, Alabama*


In attempting to construct and set up an *Avena* assay room,[3] the problem of producing and maintaining constant humidity at abnormally high levels (80–90% relative humidity) is one of primary consideration. In addition, the room must be thermostatically controlled (24°–26° C). Therefore, any method of humidification involving the use of heat, such as heating coils in a water bath, must fall within the range of the temperature tolerance (1°–2° C). In order to avoid the possible difficulty that the latter method poses, a simple, nonheating humidifying system was devised. A spray-atomizer–type humidifier, designed for a room of about 500 cu ft, was used.

The humidifier (Fig. 1 and Fig. 2, *D*) was constructed from No. 28 gauge galvanized iron, by locking and soldering. The baffle and top bracket, the latter of which holds the humidifier to the ceiling, were riveted onto the cylinder. A metal lip extends out (Fig. 1, *B* and *C*) from the front at the base of the opening. The purpose of this is to catch occasional drops of water that collect because of the direction of air flow, on the top edge of the front opening; it also serves to force the misty vapors upward, allowing for


[1] This work was supported by The Damon Runyon Memorial Fund for Cancer Research, Inc.

[2] The authors wish to extend thanks to M. Brymer, Sheet Metal Division, for assistance in construction; to George A. Reed, research engineer of Tuskegee Institute, and to M. Love, architect, for assistance in design and drawings, respectively.


[3] A darkroom for the specific bioassay determination of plant auxins, using the Went *Avena* technique.

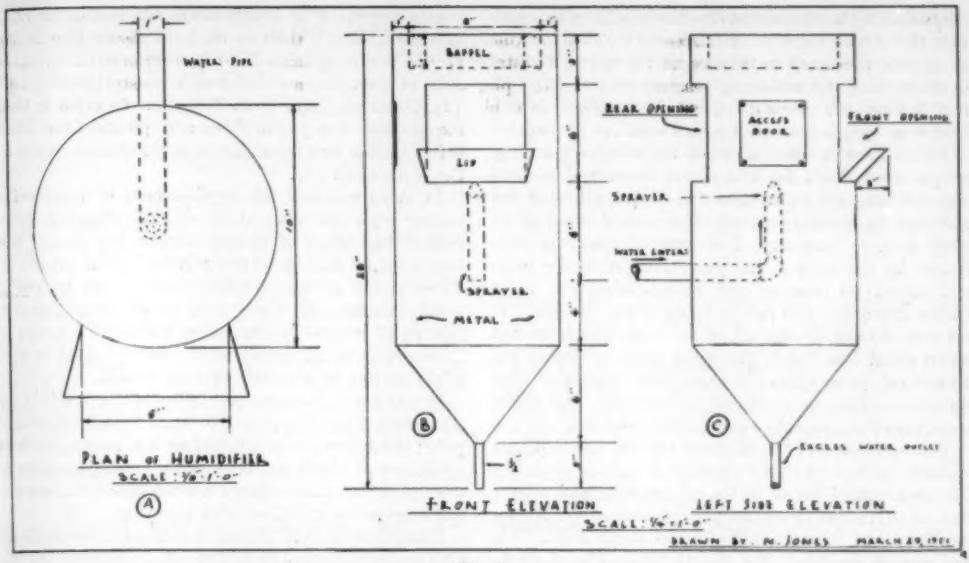


FIG. 1.

more efficient and uniform dispersion. An access door (Fig. 1, *C*) is provided for alterations or for regulating the spray while the system is in operation. This opening is made leakproof by sealing off with a rubber or cork gasket and screwing tightly into place. An excess water outlet is provided for the runoff water (Figs. 1 and 2).

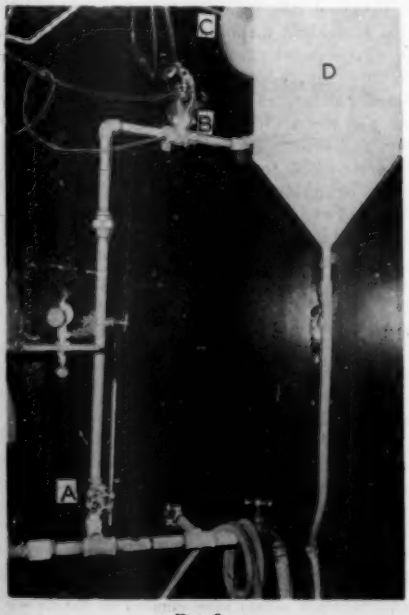


FIG. 2.

The atomizer runs from a normal wall outlet, using 3/8″ galvanized pipe with a valve (Fig. 2, *A*) (needle valve preferably) to adjust the flow. (A fine to coarse screen may be placed ahead of the valve to eliminate debris from clogging the valve.) Interspaced between the valve and the spray is a solenoid valve (Fig. 2, *B*), controlled by a Minneapolis-Honeywell humidistat (No. H63A), which maintains relative humidity within ±2%. The atomizer used in this instance is a brass jet made for an oil heating furnace at oil pressures of 100 lbs, fitted with a fine brass screen cylinder contained in a cartridge just in front of the very small aperture (*ca* 0.01″ in diam). Any atomizer-type nozzle may be used as long as the particle size of the water spray is not too large and the spray pattern is uniform and, preferably, wide-angled.

A small, 110–115 v, 8″ fan (Fig. 2, *C*), for circulating the air around the room, as well as for forcing the spray vapors out into the room, is placed about 15 in. to the rear of the humidifier. This fan, which is of the type generally used as part of the freezing unit in refrigerators, runs continuously. A similar fan (of opposite rotation for suction) is placed over the door; this fan pulls fresh air into the room by way of a louvred vent (*ca* 6″ × 8″) located near the floor on a side wall near the rear of the room and forces it out through a similar vent over the door and close to the ceiling in the front of the room. The fan is located on the outside of this latter vent. This system renews the air in the room at a relatively slow rate; as a consequence no drafts or currents occur that are obvious to the operator or that in any noticeable way affect the plants.

In this particular setup no constant temperature controls are needed, since the incoming air is already

controlled by a thermostat in the outside room such that the *Avena* room is maintained at 25° C (±1°). If it were necessary to thermostat the room, it could be done easily by installing in place of the small 8″ fan behind the humidifier, a blower-type electric heater in conjunction with a thermostatic control.

In the present situation when the relative humidity drops below 84%, the humidistat closes and magnetizes the solenoid which opens the valve, allowing the atomizer to begin spraying. The system cuts off at 88% relative humidity. The rate of spray is controlled by the valve in the water line. With the present valve type (normal gate valve), when the water comes through at full pressure (*ca* 45#), the spray is on a 4-min on, 20-min off cycle; with the valve cut down about two thirds the spray is on a 10-min on, 18-min off cycle. Thus, by controlling either the valve opening or the size of the spray aperture, one might obtain several humidity ranges and schedules.

The best index of the efficiency of this humidifying system, which next to the absence of light is probably the most critical factor in the construction and operation of an *Avena* room, is the response of the *Avena* seedlings and the coleoptiles in the assay itself. This has proved excellent. The plants follow the usual 3-day schedule and at the end of this period are between 20–30 mm tall and, most importantly, are not tough, fibrous, or brittle when pulled. A novice at the art of *Avena* assaying has run the test over a period of more than 12 weeks, involving more than 25 runs with about 120 plants per assay. The loss of plants due to decapitation or pulling of primary leaf has been less than 100 plants out of roughly 3,000, or about 3%. Originally, standards[4] of 25 and 50 γ/l were used, but it was found that 50 γ/l gave consistently limiting curvature (> 20°) and averaged 20°. The average of 11 standards at 25 γ/l was 16°, with 3 of these exceeding 20° curvature. It was decided then to use standards at 20 and 10 γ/l. These have proved satisfactory, giving an average of 16° for 20 γ/l and 10° for 10 γ/l. It is obvious that even 20 γ/l produces some limiting curvatures. This indicates good sensitivity of the plants. On this basis, it is probably valid to say that the conditions of the room, of which humidity is the prime control, are quite satisfactory.

[4] In all cases there are 12 single plants to a row; i.e., to each sample or concentration of auxin.

## Competitive Action of 2-Thiouracil and Uracil in AAF-induced Cancer of the Liver[1]

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We have shown previously that the incidence of cancer of the liver induced in rats by feeding the carcinogen 2-acetylaminofluorene (AAF) is significantly decreased by simultaneous administration of 2-thiouracil (*1*). Kidder *et al.* have shown that in the animal microorganism *Tetrahymena geleii* 2-thiouracil acts as an antagonist to uracil and inhibits growth (*2*). Mammals differ from *Tetrahymena geleii* in their requirement for pyrimidines and purines, the latter being unable to synthesize these substances, whereas the former can (*3*).

In some respects the requirements of mammalian cancer cells resemble those of *Tetrahymena geleii* rather than those of normal mammalian tissue; certain guanine analogs competitively inhibit growth of *Tetrahymena geleii* and mammalian cancer, but not of normal mammalian tissue (*4*). It was therefore considered of interest to determine whether the action of 2-thiouracil in inhibiting AAF-induced liver tumors might be due to competition with uracil.

It was not felt desirable to incorporate uracil in the diet because the acceptance of such food by the rat is poor; the animals would therefore not receive uniform quantities of either uracil or carcinogen. Consequently the uracil was administered by stomach tube in aqueous suspension stabilized with glycerin.

Kidder *et al.* (*2*) found the uracil: thiouracil inhibition index to be 100 in *Tetrahymena geleii*. On this basis, assuming the absorption of thiouracil from pellets to be 5–10 mg per day in the rat (*5*), the daily uracil requirement would be 500–1,000 mg. This amount of uracil proved to be too toxic, but 250 mg daily was tolerated by some animals, although the mortality was high even at this dosage level.

Four groups of male rats (Wistar descendants) were studied; all animals received AAF, incorporated in a cornmeal diet in a concentration of 0.03% (*6*). Group I received no additional treatment. Group II received one pellet (214 mg) of thiouracil,[2] implanted subcutaneously, every 2 weeks (*5*). Group III received 250 mg uracil by stomach tube once daily, 6 times per week. Group IV received both thiouracil pellets and uracil. This regime was continued for 90 days, after which time all animals were transferred to a stock laboratory diet (Purina dog chow). It is known that exposure to this carcinogen for 90 days is sufficient to induce carcinoma (*7*). Animals were sacrificed and examined at various intervals up to 415 days.

In a second experiment, two groups of male rats received thiouracil in drinking water (0.05%). One half the animals received 250 mg uracil by stomach tube once daily, 6 days per week; all animals received a stock laboratory diet (Purina dog chow). All were killed after 35 days, the thyroid was dissected and weighed rapidly on a Roller Smith torsion balance, fixed in Bouin solution, and examined histologically.

Animals receiving AAF alone (Group I) showed severe liver changes (Table 1). The previously reported protective influence of thiouracil (*1*) was evidenced by the occurrence of hepatoma in only one of 16 animals (Group II). Simultaneous administra-

[1] This work was supported by a grant-in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

[2] The thiouracil pellets were supplied by the Lederle Laboratories through the courtesy of St. M. Hardy.

TABLE 1

LIVER CHANGES INDUCED BY AAF; INFLUENCE OF URACIL AND THIOURACIL

| | No. animals | Duration (days) | Liver changes (hepatomas and cholangiomas) | Mean liver wt* (g/100 g body wt) |
|---|---|---|---|---|
| *Group I* AAF | 9 | 310–409 | 9 | 6.6 ± 0.71 |
| *Group II* AAF and thiouracil | 16 | 385–415 | 1 | 3.7 ± 0.23 |
| *Group III* AAF, thiouracil, and uracil | 5 | 407 | 3 | 6.5 ± 1.98 |
| *Group IV* AAF, and uracil | 5 | 385–409 | 5 | 8.2 ± 1.41 |

* Standard error = $\sqrt{\frac{\Sigma(\bar{x})^2}{n(n-1)}}$.

tion of uracil appeared to overcome this protection, 3 of 5 animals so treated showing marked liver changes (Group III). The protective action of thiouracil is reflected also in the liver weights, which indicate roughly the extent of liver changes induced by AAF. The mean liver weight of animals receiving uracil and thiouracil simultaneously (Group III) was the same as that of the animals treated with the carcinogen alone (Group I). Those given uracil and AAF (Group IV) exhibited the highest liver weights, suggesting that uracil intensifies the effect of the carcinogen on the liver.

The thyroid hyperplasia induced by thiouracil was not inhibited by simultaneous administration of uracil (Table 2). The thyroid weight and the histologic picture did not differ in the two groups.

TABLE 2

THYROID WEIGHT OF RATS TREATED FOR 35 DAYS WITH THIOURACIL, AND WITH THIOURACIL PLUS URACIL, RESPECTIVELY

| | No. animals | Mean thyroid wt (mg) | Mean thyroid wt (mg/100 g body wt) |
|---|---|---|---|
| *Group I* Thiouracil | 9 | 32.7 | 14.2 |
| *Group II* Thiouracil and uracil | 11 | 30.3 | 14.4 |

In a previous communication (*1*) the question was discussed as to whether the effect of thiouracil in counteracting the hepatic carcinogenic effect of AAF might be dependent upon the induced hypothyroidism. The present observation that uracil, in the dosage employed, does not prevent the thyroid hyperplasia induced by thiouracil, whereas it almost completely overcomes thiouracil protection against the hepatic carcinogenic action of AAF, lends additional support to the view that this "anticarcinogenic" action of thiouracil is not due to the induced hypothyroidism.

The findings suggest the possibility that uracil may be utilized by, and be a nutritional requirement for, liver cells exposed to the carcinogenic action of AAF, and that thiouracil may act as an antimetabolite under these circumstances, as it does in *Tetrahymena geleii* (*2*). Further experiments based on this working hypothesis are in progress.


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## Homologous Mechanism of Bactericidal Action and Gram-Staining


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As has recently been shown (*1*), a positive correlation exists between the affinity to wool of water-soluble substances and their antibacterial effect. Further experiments (*2*) have specified this correlation as follows: the higher the affinity to wool of a water-soluble substance at pH 7, the higher is its bactericidal action. This correlation can be demonstrated for chemically different compounds within the anion-active and the cation-active series, respectively. Finally, comparative study of 18 chemically different compounds (*3*) has led to the conclusion that the affinity to wool of a water-soluble substance is a measure of the bactericidal action against gram-positive bacteria. Some examples are given in Table 1.

Thus it is possible to predict the bactericidal effect against gram-positive bacteria of water-soluble, thermostable compounds by the determination of their affinity to wool. This determination (*3*) is carried through by treating one g of wool with 50 ml of a neutral aqueous solution of 0.2 g of the compound in question at 90° C for 10 min and weighing the wool sample before and after treatment to $10^{-4}$ g.

If wool is degraded with 0.15 *N* $Na_2CO_3$ at 80° C, first there is a diminution of the basic groups, followed by a "neutralization" of the wool proteins, and finally there is a prevalence of the acid groups. In accordance with these steps of degradation, anion-active (acid) compounds show a decrease in their affinity to wool, whereas with cation-active (basic) compounds an increase in their affinity is found.

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TABLE 1

| Substances | Affinity to 1 g wool (mM) | Relative affinities to wool | Minimum (gram +) bactericidal conc *Staph. aureus haemolyticus* | Relative bactericidal activities |
|---|---|---|---|---|
| (*a*) Anion-active | | | | |
| $CuCl_2$ | 0.0175 | 1 | 1: 25–1: 50 | 1 |
| "Eulan new" (IG) Na-salt of tetrachlor-dihydroxy-triphenylmethane-sulfonic acid | .0500 | 2.8 | 1: 240 | 6.4 |
| "Mitin FF" (Geigy) Na-salt of dichlorphenyl-(chlor/chlorsulfophenoxy/phenyl) urea; pure active ingredient | .0908 | 5.8 | 1: 200–1: 400 | 8.0 |
| $HgCl_2$* | .280 | 16.0 | 1: 400–1: 800 | 16.0 |
| (*b*) Cation-active | | | | |
| "Sapamin KW conc" (Ciba) analog of $C_{17}H_{35}$—$CO \cdot NH \cdot CH_2CH_2 \cdot N(C_2H_5)_2$ | .0282 | 1 | 1: 800 | 1 |
| "Desogen"* (Geigy) dodecyl-methyl-phenyl-trimethyl-ammonium-methosulfate | 0.0741 | 2.6 | 1: 6400–1: 12800 | 12 |

* These substances are also bactericidal against gram-negative bacteria. This fact shows that, after a high enough bactericidal activity against gram-positive forms, and also after a high enough affinity to wool value of the same substance, a correlation exists with the bactericidal activity against gram-negative bacteria also.

In this way, wool proteins[2] can be prepared which in their average IP correspond to gram-negative (pH 5) and to gram-positive (pH 2.8) bacteria, respectively (*4*). Comparing the affinity value of a compound to untreated wool (IP, pH 5) and then, for instance, to a 2-hr degraded wool-protein (pH, approx 2.8), it can be stated: The greater the first value in comparison to the second, the greater is the bactericidal effect of this compound against gram-negative bacteria as compared to its effect against gram-positive bacteria, and vice versa. This is shown in Table 2 for examples of the anion-active and cation-active series.

This indicates that the different bactericidal effects of a compound against gram-negative or gram-positive bacteria are due to its affinity to the bacterial body. Similarly, the classification of bacteria into the gram-positive or gram-negative class is based upon the different affinities of the dye gentian violet to the bacteria belonging to the two classes. This different affinity is, in its total result, mainly due to the differences in the IP in gram-negative and gram-positive bacteria. Gentian violet as a basic dye (and also applied in the gram method with phenol, for example, and thus in a weak acidic medium) is bound by the strongly acidic gram-positive bacteria (IP pH 2.8). In contrast, it is only weakly bound and afterwards washed out in gram-negative bacteria (pH 5).[3]

TABLE 2

| Substances | Affinity in mM to 1 g wool degraded with $Na_2CO_3$ (0.15 *N* at 80° C) | | Bactericidal activities against | |
|---|---|---|---|---|
| | 0 min | 2 hr | *B. paratyphi B.* (gram −) | *Staph. aureus B. haemolyticus* (gram +) |
| (*a*) Anion-active | | | | |
| "Mitin FF" pure | 0.0908 | 0.1580 | 0 | 1: 400 |
| $HgCl_2$ | .2800 | .1878 | 1: 12800 | 1: 600 |
| (*b*) Cation-active | | | | |
| "Sapamin" | .0282 | .1889 | 0 | 1: 800 |
| "Desogen" | 0.0741 | 0.1544 | 1: 600 | 1: 9600 |

There have been different attempts to explain the mechanism of gram-staining (*cf. 4*), and also the hypothesis has been advanced that gram-positiveness or -negativeness is due to differences in the IP in the two bacterial classes. From our results it is possible to predict the bactericidal activity of compounds by determination of their affinity to wool and to degraded wool with corresponding IPs of gram-negative and gram-positive bacteria. This supports the theory that not only the bactericidal action against gram-negative and -positive bacteria, but also the classification of bacteria according to Gram, is mainly based upon differences in the IP of the two bacterial classes.

[2] In this publication the comparison is made between 45- and 86-min degraded wool. The comparison between untreated and 2-hr (or even 3–4-hr) degraded wool used in the present communication is more reliable in view of the closer approximation of the IPs of untreated and about 2-hr degraded wool to those of gram-negative and gram-positive bacteria, respectively.

[3] The application of mildly oxidizing mordants in gram stains also contributes to shift the IP of gram-positive bacteria toward the acid range and thus enhances the affinity between dye and bacterium. In the case of gram-negative forms the same mordants leave the IP unaltered.

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# *News and Notes*

## Scientists in the News

**S. L. Adams** has been appointed director of research for Joseph E. Seagram & Sons, Inc. He has been associated with Seagram since 1939 and has served as assistant research director for the past two years.

Albert M. Day, director of the Fish and Wildlife Service, presented a certificate of merit to **Felipe Ancieta**, of Peru, upon the completion of his special studies in fishery subjects at the University of Michigan. Mr. Ancieta, chief of the Department of Fish Culture in the Dirección de Pesquería y Caza, arrived in this country in August 1950 to study under a training award sponsored jointly by the State Department and the Fish and Wildlife Service.

Recent visitors at the Communicable Disease Center, USPHS, Atlanta, were **Emmanuel Andreadis**, director of the Health Department, Samos District, Vathy, Samos, Greece, and **Bernard M. Clark**, deputy chief health officer, Union Health Department, Pretoria.

**C. E. Nabuco de Araujo, Jr.**, has recently been promoted from costs and operations manager to general sales manager of the Standard Oil Company of Brazil and will be in charge of all sales, operations, and marketing research activities of this company in Brazil.

**Ervin George Bailey**, past president of the American Society of Mechanical Engineers and vice-president of Babcock & Wilcox Company, has been selected by the Board of Award to receive the 1952 John Fritz Medal and Certificate "for outstanding engineering achievements in the field of combustion and distinguished service to his fellows in advancing the engineering profession." The medal, established in 1902 by friends of John Fritz on his eightieth birthday to honor him for his great contributions in the manufacture of steel and in the advancement of industry generally, is perpetuated by the four leading professional engineering societies, ASCE, AIME, ASME, and AIEE, as a joint honor for scientific or industrial achievement in any field of pure or applied science, without restriction on account of nationality or sex.

The Committee for the Foundation for the Study of Cycles has elected **Harold G. Bowen**, USN (Ret.), a member. Admiral Bowen is executive director of the Thomas Alva Edison Foundation in West Orange, N. J.

Among recent visitors at the Kessler Institute for Rehabilitation, West Orange, N. J., were **Claudia D. Bradley**, of the Spastic Center for Children, Sidney, Australia; **Harold Balme**, of Kent, England, adviser on rehabilitation to the UN; and **Soledad Bermejo**, of the National Orthopedic Hospital in Mandaluyong, Rizal, P. I.

**J. M. Burgers**, director, Laboratorium voor Aerodynamica, Delft, recently delivered a series of lectures on the flow of gases in small tubes at the National Bureau of Standards. **S. R. de Groot**, professor of theoretical physics, Utrecht University, was another Dutch visitor, and **A. F. A. Harper**, Division of Physics, CSIR, Australia, visited laboratories and discussed research in temperature measurements, cryogenics, and thermodynamics.

**Harold B. Hoskins**, president of the Board of Trustees of the American University of Beirut, has been appointed president of the Near East College Association, which represents the American College for Girls and Robert College, Istanbul; the American University of Beirut and International College, Lebanon; Athens College and Anatolia College, Greece; and Damascus College, Syria. He served recently as a special consultant to George C. McGhee, Assistant Secretary of State for Near Eastern and American Affairs, and is on the point of joining the State Department to give full-time attention to Near and Middle Eastern problems.

**H. E. Humphreys, Jr.**, president of the United States Rubber Company, has been elected to the additional office of chairman of the board. He will succeed **Herbert E. Smith**, chairman and former president, who has retired after 38 years of service but will continue as a director and member of the finance committee.

**E. P. Killip**, head curator of the Department of Botany of the U. S. National Museum, Washington, D. C., until his retirement this year, has been elected research associate in phanerogamic botany at the Chicago Natural History Museum.

**Donald C. Martin** has been appointed head of the Department of Physics at Marshall College, Huntington, W. Va., succeeding **Ralph P. Hron**, who has retired.

**Martin A. Mason**, chief of the Engineering and Research Branch and chief engineer of the Army's Beach Erosion Board, has been named dean of the School of Engineering, George Washington University. He succeeds **Frederick M. Feiker**, who becomes professor emeritus of engineering administration in residence.

**Peter G. Meek** has been appointed acting director of the Commission on Chronic Illness, to replace **Morton L. Levin**, who directed the commission during a leave of absence from the New York State Department of Health. Dr. Levin has been recalled to his position as assistant commissioner for medical services.

**David H. Ross** has been elected president and general manager of Gair Company Canada Limited, Toronto, a wholly owned subsidiary of the Gair Company, of New York. Mr. Ross was previously a vice president, as well as a director, of Gair Canada. **W. George Cowan** was elected vice president, director, and assistant to the president. **Alpine L. Mitchell** has been made a vice president and director, and **J. Stanley Babbitt** has been made a director and designated vice president in charge of Montreal operations.

**Charles E. Schaffner** has been appointed to succeed **Cornelius Wandmacher** as director of the evening session devoted to the engineering curriculum at the Polytechnic Institute of Brooklyn. The Polytechnic evening session has an enrollment of more than 2,000 undergraduate students in its winter and summer sessions. Professor Schaffner became a member of the Polytechnic faculty, on which he is an associate professor of civil engineering, in 1946. Dr. Wandmacher will become head of the Department of Civil Engineering at the University of Cincinnati.

**Frederic V. Schossberger,** Czechoslovakian scientist, has joined the chemistry department of Armour Research Foundation of Illinois Institute of Technology as a research physical chemist. He was formerly a research chemist with the Glidden Corporation, Baltimore.

**Charles A. Shaunesey, Jr.,** has assumed command of the QM Food and Container Institute for the Armed Forces, succeeding **Joseph Kujawski,** now assigned to duty as chief, Food Service Division, Office of the Quartermaster General. Col. Shaunesey comes to the Institute from a four-year tour of duty at the New York QM Industrial Mobilization Planning Office. Among other staff changes, **Raymond R. Guehring** has taken charge of the Military Operations Office of the institute, succeeding **Ewing Elliott,** now filling an overseas assignment. **Carl S. Pederson** was appointed head of the Stability Division, succeeding **Harry Fevold,** now with the Baxter organization. In joining the institute staff, Dr. Pederson concluded a long association with the New York State Agricultural Experiment Station of Cornell University, where he held a professorship in bacteriology. **Virgil O. Wodicka** has been named assistant to the scientific director, Dr. Tressler. He has been working on nutritional studies and quality control with Libby, McNeill & Libby since 1948.

**Esmond E. Snell** has rejoined the University of Texas Biochemical Institute staff as professor of chemistry. Dr. Snell was on the staff from 1939 to 1945, then joined the University of Wisconsin faculty.

**Emanuel Stein** has been appointed chairman of the Department of Economics at New York University's Washington Square College of Arts and Science. He has been a member of its faculty since 1930.

**Philip R. Tarr,** of St. Louis, has been named assistant to the president of Monsanto Chemical Company. Mr. Tarr joined Monsanto in 1946, and in 1950 he was named European technical representative, but his appointment was cancelled two months later in order that he could be named assistant director of industrial preparedness.

**Henry S. Thomas** has been appointed assistant medical director of Sharp & Dohme. Since his release from the U. S. Army Air Force as command flight surgeon in 1946, he has been medical director of Ciba Pharmaceutical Products, Inc., and of the Nepera Chemical Company.

**C. D. W. Thornton** has joined the Operations Analysis Staff of the general manager of the AEC. He was formerly technical assistant in the Source & Fissionable Materials Accountability Branch of the Division of Production. He will continue as chairman of the Fissionable Standard Samples Committee of the AEC.

The University of Chicago Press announces the appointment of **Ilza Veith** as assistant editor in biology and medicine. Mrs. Vieth is assistant professor of the history of medicine in the Departments of Medicine and History at the university. Since 1947 she has been consultant in Oriental medicine at the Surgeon General's Office, U. S. Army Medical Library. Mrs. Veith will work with books published under the auspices of the University Committee on Publications in Biology and Medicine, as well as with other Press publications in the medical and biological fields.

**Enrico Via,** chief of Radiological Service of the Cancer Institute of Rome, is spending six months in this country under the auspices of WHO. Dr. Via is studying the use of radioisotopes in medicine and is dividing his time between the Medical Division of the Oak Ridge Institute of Nuclear Studies and Memorial Hospital in New York City.

**Paul E. Waggoner** has been appointed to the staff of the Plant Pathology Department of the Connecticut Agricultural Experiment Station. Dr. Waggoner will conduct research on the effect of atomic radiation on plant disease, under the station's contract with the AEC and will initiate work on the spread of plant diseases. Dr. Waggoner has been with the Division of Disease Survey, Bureau of Plant Industry, and was stationed at Iowa State College.

**A. C. Walker,** of the Bell Laboratories, has been awarded the Louis Edward Levy medal of the Franklin Institute, "in recognition of his outstanding paper, 'Growing Piezoelectric Crystals,' appearing in the December 1950 issue of the *Journal of the Franklin Institute.*"

President Truman has accepted the resignation of **William Webster** as chairman of the Research and Development Board of the Department of Defense. **Walter G. Whitman,** of MIT, has been named to succeed him. Mr. Webster, who has served in research and atomic energy posts, has been on leave as executive vice president of the New England Electric System of Boston. He has served in the present post since March 1950. Dr. Whitman, professor of chemical engineering at MIT, was director of the Lexington project of the Atomic Energy Commission in 1948. **Norman L. Winter,** of Sperry Gyroscope Company, has been appointed chairman of the Navigation Technical Group of the Research and Development Board.

## Education

**Case Institute of Technology** will offer a graduate program of study in engineering administration beginning with the fall semester. Human relations, com-

munication within industry, and external relations of a company with its community and the government will be stressed.

The **Council on Postgraduate Medical Education** and the New York state chapter of the **American College of Chest Physicians,** cosponsors of the annual postgraduate course in diseases of the chest, will present the course at the Hotel New Yorker, New York City, Nov. 12–17. Tuition is $50, and the number of registrants will be limited. Send applications to the American College of Chest Physicians, 112 E. Chestnut St., Chicago 11, Ill.

An expedition sponsored by the **Council for Scientific and Industrial Research** left Johannesburg, South Africa, last month for the Kalahari Desert to investigate why the teeth of primitive Bushmen remain so sound as compared with those of white men. A. J. Clement, whose studies of dental decay have been supported by CSIR, a nutritionist from the Institute for Medical Research, and William Rae, bacteriological technician, are among members of the expedition.

A Second Physical Institute, which will be concerned with the study of nuclear physics and cosmic rays, has been established at the **University of Heidelberg.** Otto Haxel, formerly of Göttingen, is director.

A series of lectures and demonstrations in the biophysical aspects of virology will be given Sept. 20–22 at the **University of Illinois** by Max A. Lauffer, of the University of Pittsburgh. Part of the Gehrmann lecture series sponsored by the Department of Bacteriology, the individual lectures will deal with ultracentrifugation, electrophoresis, and electron microscopy. All who wish to attend should register before *Sept. 15* with J. E. Kempf, University of Illinois, 808 S. Wood St., Chicago 12, Ill.

Seven French and 101 British teachers have arrived in the U. S. as exchange teachers in 32 states, in the program sponsored for the sixth year by the **Office of Education,** FSA, in cooperation with the State Department. The American teachers participating in the program sailed for Europe on July 26. Interchanges are also made with Canada, Belgium, Luxemburg, Norway, the Netherlands, Austria, Italy, Australia, and New Zealand.

The **University of Texas Medical Branch,** Galveston, will offer graduate study in the fields of anatomy, biochemistry and nutrition, microbiology, pharmacology, and physiology for the first time this year. The courses will be correlated with opportunities for study at Austin and in coordination with Southwestern Medical School, Dallas, the M. D. Anderson Hospital for Cancer Research, Houston, and the University of Texas School of Dentistry.

## Grants and Fellowships

The **American Dermatological Association** will award a cash prize to the writer of the best essay submitted for original work, not otherwise previously published, relative to some fundamental aspect of dermatology or syphilology. The prize-winning candidate may be invited (with expenses paid) to present his paper before the annual meeting of the association in Colorado Springs next April. Deadline for papers is *Dec. 1.* For full details write to Louis A. Brunsting, 102 Second Ave., S. W., Rochester, Minn.

Sixty new grants-in-aid, amounting to $311,912, have been approved for various institutions by the **American Heart Association,** in addition to $166,750 allocated earlier this year. Largest of the new awards ($26,334) went to Western Reserve University School of Medicine, for projects under the direction of Edward H. Bloch, Arnold Lazarow, and Carl J. Wiggers. Next largest amounts went to Columbia University for studies by Stanley E. Brady, Ralph A. Deterling, Jr., and Ferdinand F. McAllister.

Walter L. Magee, of Brooklyn, is the recipient of a fellowship from the **Icelandic-American Society,** Reykjavik, and the **University of Iceland** for study in Iceland during 1951–52, designed to promote cultural understanding between the U. S. and Iceland.

**International Council of Scientific Unions** and **Unesco** will soon publish rules for an international annual prize of £1,000 ($2,800) for the best works of scientific popularization. The first award will be made in 1952. Further information can be obtained from Unesco, 19 Ave. Kleber, Paris[xvi].

Werner I. Frank, of Brookline, Mass., a student in electrical engineering at MIT, has been awarded a four-year university scholarship by **RCA Institutes.** Examination of the candidates was made by a committee consisting of Charles E. Skinner, Walter A. Curry, and George L. Van Deusen. The winner is a native of Switzerland, who has become a U. S. citizen.

John E. Pfeiffer, free-lance science writer, has been awarded a fellowship by the **Eugene F. Saxton Memorial Trust** for the completion of a popular book about the brain. The trust was established in 1943 in memory of Harper's chief literary editor, to provide assistance to creative writers.

First awards from the **USPHS Microbiological Institute,** covering a wide range of projects, number 102 and amount to more than a million dollars. Juan A. Montoya, of the Pan American Sanitary Bureau, Guatemala City, received the largest ($47,150), for a study of onchocerciasis; other large awards went to the California State Department of Health ($38,845) for work on Q-fever, to the Leonard Wood Memorial ($34,085) for research on leprosy, and to the Trudeau Foundation ($25,596) for a study of streptomycin-resistant tubercle bacilli.

Deans of medical schools in the U. S. and Canada are invited by the **John and Mary R. Markle Foundation** to make nominations for Scholars in Medical Science on or before *Dec. 1.* Grants will be made only to candidates holding or expecting to hold a full-time

faculty appointment on the staff of a medical school. Grants of $30,000, payable at the rate of $6,000 annually, are made to the schools, each of which may nominate one candidate. For full information write to the foundation at 14 Wall St., New York 5.

## Recent Deaths

**W. E. Agar** (69), zoologist, Australia, July 14; **Clifford C. Anderson** (81), archaeologist, Xenia, Ohio, Aug. 8; **Cyril Ashford** (83), educator, Reading, Eng., Apr. 29; **George H. Ashley** (84), geologist, Harrisburg, Pa., May 28; **William M. Bacon** (82), mechanical engineer, Cleveland, Aug. 15; **William J. Baker** (50), chief research and acting head, Oregon Forest Products Laboratory, Portland, Aug. 10; **Boris A. Bakhmeteff** (71), civil engineer, Brookfield, Conn., July 21; **Albert C. Barnes** (78), chemist, Chester Co., Pa., July 24; **Donald S. Bartlett** (57), dentist, Waukegan, Ill., Aug. 13; **Harold H. Bender** (69), etymologist, Princeton, N. J., Aug. 15; **Philip Berke** (44), anesthetist, Freeport, L. I., July 29; **Paul Bonnot** (52), fishery and marine biologist, Bodego Bay, Calif., Aug. 1; **Claude S. Bryan** (43), veterinarian, Ann Arbor, Mich., July 30; **Richard S. Buck** (86), civil engineer, Washington, D. C., Aug. 1.

**Bert Caldwell** (76), physician, Beloit, Wis., July 26; **Alfred C. Callen** (63), mining engineer, Ocean City, N. J., July 30; **Aimé A. Cotton** (81), physicist, Sèvres, France, Apr. 16; **James F. Couch** (62), chemist, Philadelphia, Aug. 9; **Harrison W. Craver** (75), librarian, Baltimore, Md., July 26; **Lawrence Crawford** (84), mathematician, Cape Town, April 4; **John W. Croskey** (93), ophthalmic surgeon, Philadelphia, July 30; **Fred B. Cutter** (79), consulting engineer, Newton Center, Mass., Aug. 14; **Hugh L. Davis** (56), chemist, Chicago, Aug. 17; **Will C. Davis** (38), dermatologist and syphilologist, Portland, Ore., June; **William H. Davis** (64), pharmaceutical chemist, White Plains, N. Y., July 29; **Clive Day** (80), political economist, Greensboro, Vt., July 27; **James H. Dempster** (78), roentgenologist, Detroit, Aug. 5; **Horace St. John K. Donisthorpe**, taxonomist and myrmecologist, Apr. 22; **Clarence E. Field** (81), cancer expert, Highlands, N. J., Aug. 3; **Earl K. Fischer** (46), physical chemist, Kensington, Md., Aug. 3; **Frank L. Fullam** (81), explosives expert, Princeton, N. J., July 31; **Chester A. Fulton** (67), mining engineer, Baltimore, Md., Aug. 16.

**Samuel Goldschmidt** (64), physiologist, Philadelphia, Aug. 8; **George H. Hamlin** (100), consulting engineer, Orono, Maine, July 17; **George B. Hassin** (79), neurologist, Chicago, Aug. 15; **J. L. R. Hayden** (70), engineer, Schenectady, N. Y., Aug. 12; **Lucy S. Hertzog** (82), homeopathist, Cleveland, Aug. 11; **Jan Hirschler**, cytologist, Gdansk-Wrzeszcz, Poland, Jan. 3; **H. A. Humphrey** (82), chemical engineer, South Africa, Mar. 9; **Arthur E. Hutchinson** (71), engineer and air pollution specialist, Cleveland, Aug. 18; **S. Orie Johnson** (70), conservationist, Berkeley, Calif., Aug. 5; **William C. Johnson** (49), industrialist, Milwaukee, Wis., July 26; **Wilbur Judson** (71), mining engineer, New York, Aug. 9; **W. Wallace Kellett** (60), autogiro pioneer, Philadelphia, July 22; **William H. Kiekhofer** (68), economist, Madison, Wis., Aug. 1; **Jerome Kohout** (63), solid fuels specialist, Baltimore, Md., Aug. 9; **Alan P. Lee** (61), consulting chemist and engineer, Morristown, N. J., Aug. 9; **Marie Louise LeFort** (76), physician, Maplewood, N. J., Aug. 6.

**Francis X. McGovern** (57), physician, Washington, D. C., July 28; **Niles Martin** (66); physician, Philadelphia, July 28; **Florence L. Meredith** (63), hygienist and physician, Watertown, Mass., Aug. 16; **Cyrus W. Miller** (55), refrigeration engineer, North Tarrytown, N. Y., Aug. 13; **Kingo Miyabe** (90), botanist and founder Hokkaido College, Japan, Mar. 16; **Grigori I. Nosov** (46), industrialist, Moscow, announced Aug. 10; **Frank O'Donnell** (71), weather expert, Toronto, July 23; **William B. Palmer** (96), civil engineer, Bridgeport, Conn., July 22; **John B. Pastore** (46), hospital official, Pelham, N. Y., Aug. 18; **James E. Paullin** (69), physician, Atlanta, Ga., Aug. 13; **Harry B. Pearson** (65), engineer, New York, Aug. 11; **David W. Peters** (61), educator, Blacksburg, Va., Aug. 2; **Myles Purvin** (77), physician, Long Beach, Calif., July 27.

**Gordon Reel** (80), aviation engineer, New York, Aug. 18; **Martin A. Rosanoff** (76), chemist, Pittsburgh, July 30; **Edward A. Ross** (84), sociologist, Madison, Wis., July 22; **Wilfred N. St. Peter** (69), physicist, Pittsburgh, July 20; **Ferdinand Sauerbruch** (75), surgeon, Berlin, July 2; **Burlingham Schurr** (66), naturalist, Granby, Mass., July 12; **Reuben Seid** (53), optometrist, Chicago, July 8; **R. Seton-Watson** (71), political historian, Isle of Skye, July 25; **M. Mortimer Sherman** (62), psychiatrist, Brooklyn, N. Y., June 21; **William M. Shoemaker** (56), mechanical engineer, Philadelphia, July 30; **Ivan V. D. Shunk** (60), botanist, Raleigh, N. C., July 11; **James S. Thompson** (51), physicist, Chicago, Aug. 5; **W. W. Thomson** (54), scientific editor, Ottawa, Canada, Apr. 28; **Paul Titus** (66), obstetrician and gynecologist, Pittsburgh, June 28; **D. F. Twiss** (68), rubber chemist, England, May 23; **Louis E. Underwood** (73), engineer, Wilmington, N. Y., July 1; **Benjamin D. White** (84), agriculturist, Maquoketa, Iowa, July 6; **Arthur B. Williams** (71), naturalist, Cleveland, Aug. 18; **Arthur M. Wilson** (51), chemist, Sussex, N. J., July 22; **Leroy A. Wilson** (50), AT&T president, New York, June 28; **George F. Zook** (66), educational leader, Arlington, Va., Aug. 17.

Entries in the **Fifth International Photography-in-Science Salon**, sponsored annually by THE SCIENTIFIC MONTHLY and the Smithsonian Institution, should be sent (prepaid) to the Editor, *The Scientific Monthly*, 1515 Massachusetts Ave., N.W., Washington 5, D. C., Nov. 1–Nov. 27. All accepted photographs will be shown at the AAAS meeting in Philadelphia Dec. 26–31, and later throughout the U. S. For entry blanks and full information write to the address given above.

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# *Publications Received*

*Plankton Copepods from the Atlantic Sector of the Antarctic.* W. Vervoort. Verhandel. Koninkl. Nederland. Akad. Wetenschap., Afdeel. Natuurk., Sect. II, 47 (4). *Studies on the Sāmaveda.* Pt. I. Barend Faddegon. Afdeel. Letterk., 57 (1). North-Holland Pub. Co., Amsterdam. 1951.

*Recherches Geologiques et Minieres aux Iles Saint-Pierre et Miquelon.* E. Aubert de la Rüe. Office de la Recherche Scientifique Outre-Mer, Paris. 1951.

*Research at Northwestern University.* A Report Published by the Faculty Committee on Research. Northwestern Univ. Information Vol. XIX, No. 33. Northwestern Univ. Press, Evanston, Ill. April 16, 1951.

*Semi-Annual Report to Stockholders 1951.* National Research Corporation, Cambridge, Mass. 1951.

*Austenitic Grain-Size Control of Steel.* B. R. Nijhawan and A. B. Chatterjea. Pub. Div., Council of Scientific & Industrial Research, 20 Pusa Road, New Delhi 5. 1951. Rs.3/–.

*Bausch & Lomb Dynoptic Labroscopes.* Catalog D-185. Bausch & Lomb Optical Co., Rochester 2, N. Y. August 1951.

*A Citizen's Handbook of Sexual Abnormalities and the Mental Hygiene Approach to Their Prevention.* A Report to the Committee on Education of the Governor's Study Commission on the Deviated Criminal Sex Offender, State of Michigan. Samuel W. Hartwell. 1950.

*Cretaceous and Eocene Peduncles of the Cirripede Euscalpellum.* Geology, Vol. 1, No. 5. Thomas H. Withers. 5s. *Some Jurassic and Cretaceous Crabs. (Prosoponidae).* Geology, Vol. 1, No. 6. T. H. Withers. British Museum (Natural History), London 1951. 5s.

*French Bibliographical Digest. Physics.* No. 7. Cultural Division, French Embassy, 934 Fifth Ave., New York 21. 1951. Free.

*Frontiers in Space.* Publication of the Mount Wilson and Palomar Observatories. California Institute of Technology Bookstore, Pasadena. 1951. 50¢.

*High-Calcium Limestones in the B & O Area.* Baltimore and Ohio Railroad Co., Baltimore.

*Die Körperproportionen und Ihre Veränderungen im Kleinkindalter.* Inaugural dissertation. von Theodor Müller. Anthropologisches Institut der Universität Zürich, Zürich, Switzerland. 1950.

*Irrigation Waters of Utah.* Bulletin 346. J. P. Thorne and D. W. Thorne. June 1951. *The Life History and Management of the Mountain Whitefish* Prosopium Williamsoni (Girard) *in Logan River, Utah.* Bull. 347. William F. Sigler. May 1951. *Cost and Efficiency of Producing Canning Corn in Cache County, Utah, 1949.* Bull. 348. Earnest M. Morrison and Willis G. Kearl. June 1951. *Management of Irrigation and Drainage Enterprises in Utah.* Bull. 349. J. Howard Maughan and O. W. Israelsen. June 1951. *Cemeteries of Summit Counties.* Bull. 350. Joseph A. Geddes and Carmen Fredrickson. June 1951. *How Serious Are the Defects of Mechanical Blocking of Sugar Beets?* Cir. 128. D. W. Pittman. June 1951. *Utah's Land Resources.* Spec. Rept. 4. Lawrence A. Reuss and George T. Blanch. Agricultural Experiment Station, Utah State Agricultural College. June 1951.

*Major Activities in the Atomic Energy Programs, January–June 1951.* U. S. Atomic Energy Commission. GPO, Washington, D. C. July 1951. 35¢.

# Longmans books

Jones

## GENERAL ASTRONOMY

By Sir Harold Spencer Jones, Astronomer Royal. Third edition. Ready, autumn 1951. 404 pages. 115 line drawings and 31 plates. About $5.75. This new edition has been thoroughly revised and corrected according to recent observations. It remains a non-mathematical treatment of the subject.

Cohen & Rogers

## GAS TURBINE THEORY

By H. Cohen, University of Durham, and G. F. C. Rogers, Bristol University. Ready, September 1951. 288 pages. Line illustrations in text. About $5.75. A discussion of the basic and well established theories of the gas turbine, including only those subjects directly applicable to its practical design.

Cowie

## POTASH: Its Production and Place in Crop Nutrition

By G. A. Cowie, Chief Technical Adviser to Potash, Ltd. Ready, autumn 1951. 176 pages. 8 plates. About $4.00. This book deals with the use of potash as plant food, not only in England, but in other countries as well. It contains a survey of world potash deposits, and a chapter on the processes of manufacture of salts required for agricultural purposes.

Lang

## SOME ASPECTS OF FLUID FLOW

Edited by H. R. Lang, Secretary and Editor of the Institute of Physics, London. Ready. 296 pages. 112 figures. Tables and bibliographies. $9.50. Papers presented at the Institute conference of October 1950, with reports of discussions; covers fundamental problems, techniques, and applications of present knowledge and techniques.

Sparkes & Smith

## CONCRETE ROADS

By F. N. Sparkes, Senior and Principal Officer and Head of the Concrete Section, Road Research Laboratory, and A. F. Smith, Assistant County Surveyor, Surrey. Roadmakers Library, Vol. XI. Ready, autumn 1951. 480 pages. 246 illustrations. About $11.50. This volume deals with all aspects of the subject from the consideration of the subsoil to the laying of the concrete; also discusses the best type of machinery to use.


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# *Meetings & Conferences*

Sept. 10–12. American Institute of Biological Sciences (Annual). University of Minnesota, Minneapolis.

Sept. 10–12. Mycological Society of America. University of Minnesota, Minneapolis.

Sept. 14–15. International Union of Pure and Applied Chemistry. Washington, D. C.

Sept. 17–21. Conference on Industrial Experimentation. Columbia University, N. Y.

Sept. 18. International Symposium on Chemistry of ACTH, sponsored by Armour and Company. Palmer House, Chicago.

Sept. 25–28. American Roentgen Ray Society. Washington, D. C.

Sept. 25–28. American Society of Mechanical Engineers (Fall). Hotel Radisson, Minneapolis.

Sept. 26–28. National Metal Trades Association. Palmer House, Chicago.

Sept. 29. Society for Clinical and Experimental Hypnosis (Annual). New York Academy of Sciences, New York.

Sept. 29–Oct. 5. American Society for Metals. Detroit, Mich.

Sept. 29–Oct. 5. American Welding Society. Detroit, Mich.

Oct. 1–3. Association of Official Agricultural Chemists. Shoreham Hotel, Washington, D. C.

Oct. 3–4. Association of American Feed Control Officials. Shoreham Hotel, Washington, D. C.

Oct. 3–5. American Institute of Mining and Metallurgical Engineers (Annual). Oklahoma Biltmore, Oklahoma City.

Oct. 3–6. National Society for Crippled Children and Adults (Annual). Palmer House, Chicago.

Oct. 4–6. American Physical Society, Division of Electron Physics. Conference on Gaseous Electronics, G-E Research Laboratory, Schenectady, N. Y.

Oct. 5. American Crystallographic Association, Michigan chapter. University of Michigan, Ann Arbor.

Oct. 5. Association of American Fertilizer Control Officials. Shoreham Hotel, Washington, D. C.

Oct. 5–6. Ohio Mineral Industries Conference (Annual). Ohio State University, Columbus.

Oct. 6. Association of Economic Poison Control Officials. Shoreham Hotel, Washington, D. C.

Oct. 8–10. American Forestry Association and the Society for the Protection of New Hampshire Forests (Annual). Jefferson, N. H.

Oct. 8–10. The American Oil Chemists' Society (Fall). Edgewater Beach Hotel, Chicago.

Oct. 8–12. National Safety Council. Stevens Hotel, Chicago.

Oct. 9–11. American Meteorological Society. Minneapolis.

Oct. 10–12. Porcelain Enamel Institute. Ohio State University, Columbus.

Oct. 15–17. American Gas Association (Annual). Kiel Auditorium, St. Louis.

Oct. 15–18. American Dental Association (Annual). Washington, D. C.

Oct. 15–19. National Metal Congress and Exposition. Detroit.

Oct. 15–19. Society of Motion Picture and Television Engineers (Fall). Hollywood Roosevelt Hotel, Hollywood, Calif.

Oct. 15–19. World Metallurgical Congress (Annual). Detroit, Mich.

Oct. 18–20. National Association of Corrosion Engineers (Regional). Corpus Christi, Tex.

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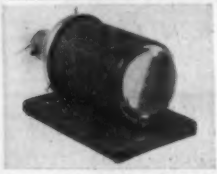

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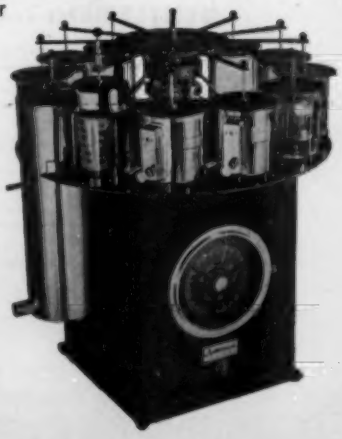

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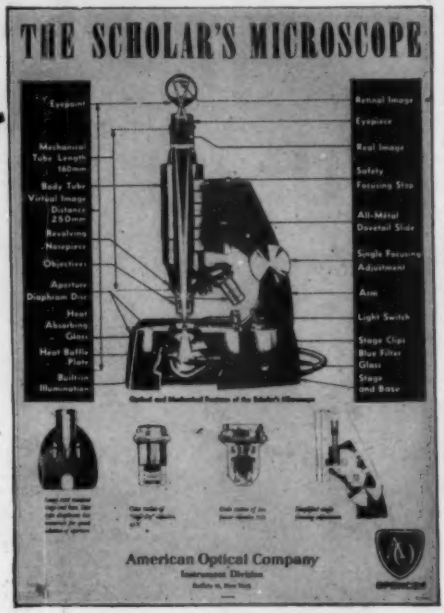


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